Tri-institutional Effort to Probe Cyanobacterial Metabolic Overflow: From Research to Training

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Understanding energy metabolism in cyanobacteria has potential to guide new strategies for biofuel and chemicals production. Metabolite overflow is an alternative energy dissipation mechanism identified in a glycogen mutant of cyanobacteria (Cano et al 2018). Recently, the deletion of the polyphosphate kinase gene (\$\Delta ppk\$) increased laboratory productivity by re-wiring energy distribution (Sebesta et al 2024). We hypothesize that the metabolite overflow plays a dynamic role in carbon sink and energy management in cyanobacteria. Here we present data in metabolite overflow in the cyanobacterium \$\Synechocystis\$ 6803 and \$\Synechococcus\$ 7942. (1) HPLC and LCMS analysis showed that WT Synechocystis can overflow alpha-ketoglutarate (AKG) and pyruvate in low quantities at normal growth conditions. (2) Mutant Synechocystis with the polyphosphate deletion maintains robust Ci fixation capability and secrets about three-fold more AKG and pyruvate than the WT strain. (3) HPLC and FTIR analysis showed that the growth medium of \$\Synechocystis\$ 6803 has different time-dependent overflow metabolite profiles. Distinctive time-dependent IR profiles in the cells of \$\Synechocystis\$ 6803 and \$\Synechococcus\$ 7942 were also observed. The preliminary lines of evidence support that the metabolite overflow

may be an energy management strategy in cyanobacteria and may have potential applications in the forensic community. The use of such research activities in teaching at Alabama State University will be discussed (Figure 1). This work was supported by the U.S. Department of Energy, Office of Science Energy Earthshot Initiative, as part of the Science Foundations for Energy Earthshot (DOE SFEE) Projects under grant number DE-SC0024702.

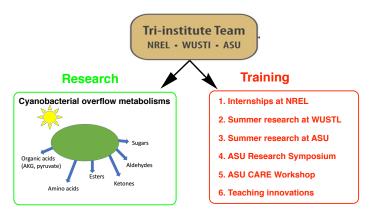


Figure 1. Joint efforts of the tri-institutional team (NREL-WUSTL-ASU) to probe cyanobacterial metabolic overflow. (a) fundamental research (left) and (b) training and teaching (right).

Cano et al (2018) Manipulation of glycogen and sucrose synthesis increases photosynthetic productivity in cyanobacteria, Front Microbiol. 14: 1124274.

Sebesta et al (2024) Polyphosphate kinase deletion increases laboratory productivity in cyanobacteria. *Front. Plant Sci.* 15:1342496. doi: 10.3389/fpls.2024.1342496